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4/1/86

Date of Signature

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of )

Richard H. Tullis )

Serial No. 06/314,124 )

Filed: October 23, 1981 )

For: OLIGONUCLEOTIDE THERA- )  
PEUTIC AGENT AND )  
METHOD OF MAKING SAME )

Group Art Unit: 127

Examiner: J. Martinell GROUP 120

Los Angeles, CA 90010  
March 31, 1986

RECEIVED

APR 15 1986

AMENDMENT

Hon. Commissioner of Patents  
and Trademarks  
Washington, D. C. 20231

Sir:

Responsive to the Office Action mailed November 27,  
1985, please amend the above-identified application as  
follows:

IN THE CLAIMS

Cancel claims 1, 3-28, and 30-39 without prejudice.

Substitute therefor the following claims:

--54. A method of developing oligodeoxyribo-  
nucleotide therapeutic agents for use in in vivo inhibition  
of the synthesis of one or more targeted proteins in a cell  
without substantially inhibiting the synthesis of  
non-targeted proteins, comprising the steps of:

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determining the base sequence of an organism's messenger ribonucleic acid, said base sequence coding for at least a portion of said protein targeted for inhibition<sup>and</sup>

7  
10 synthesizing an oligodeoxyribonucleotide, the nucleotide sequence of which is substantially complementary to at least a portion of said base sequence, ~~and~~

7  
15 at least a portion of said oligodeoxyribonucleotide <sup>in the form of a phosphotriester</sup> being ~~a more stable form~~ in order to limit degradation in vivo, whereby said oligodeoxyribonucleotide may be introduced into the cells of said organism for hybridization with said messenger ribonucleic acid base sequence coding for at least a portion of a protein targeted for inhibition so as to substantially block translation of said base sequence and inhibit synthesis of said targeted protein.--

--55. The method of ~~claim 54~~, wherein said more stable form is a phosphotriester form.--

--56. The method of claim 54, wherein said oligodeoxyribonucleotide comprises at least 14 nucleotides.--

--57. The method of claim 54, wherein said oligodeoxyribonucleotide comprises about 23 nucleotides.--

--58. The method of claim 54, wherein the order of said base sequence is determined from ribonucleic acid or

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deoxyribonucleic acid coding for said targeted protein prior to synthesizing the oligodeoxyribonucleotide.--

--59. The method of claim 54, wherein the order of said base sequence is determined from messenger ribonucleic acid coding for said targeted protein prior to synthesizing said oligodeoxyribonucleotide.--

--60. The method of claim 54, wherein the order of said base sequence is determined from said targeted protein prior to synthesizing said oligodeoxyribonucleotide.--

--61. The method of claim 54 further comprising the step of inserting said oligodeoxyribonucleotide into a plasmid for cloning.--

--62. The method of claim 61, wherein said plasmid is pBR322.--

--63. The method of claim 62, wherein said oligodeoxyribonucleotide is inserted into said plasmid with a linker base sequence.--

--64. The method of claim 63, wherein said linker base sequence is GATTCGAATC or CTAAGCTTAG.--

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--65. The method of claim 63, wherein said linker base sequence is susceptible to partial degradation by Hind III or Alu I restriction nucleases.--

--66. The method of claim 54, wherein said oligodeoxyribonucleotide is synthesized chemically.--

--67. The method of claim 54 further comprising the step of:

cross-hybridizing the oligodeoxyribonucleotide against messenger ribonucleic acid from at least one species different from said organism, and selecting that fraction of the oligodeoxyribonucleotide which does not so hybridize so as to increase the specificity of the selected oligodeoxyribonucleotide to messenger ribonucleic acid unique to said organism.--

--68. A method of selectively inhibiting in vivo synthesis of one or more specific targeted proteins without substantially inhibiting the synthesis of non-targeted proteins, comprising the steps of:

5 synthesizing an oligodeoxyribonucleotide having a nucleotide sequence substantially complementary to at least a portion of the base sequence of messenger ribonucleic acid coding for said targeted protein,

10 at least a portion of said oligodeoxyribonucleotide being <sup>in the form of a phosphotriester</sup> a more stable form to limit degradation in

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vivo;

introducing said stable oligodeoxyribonucleotide  
into a cell; and

15 hybridizing said stable oligodeoxyribonucleotide  
with said base sequence of said messenger ribonucleic acid  
coding for said targeted protein, whereby translation of said  
base sequence is substantially blocked and synthesis of said  
targeted protein is inhibited.--

<sup>2</sup>  
~~--69.~~ The method of claim <sup>1</sup>~~68~~, wherein said  
oligodeoxyribonucleotide comprises at least 14 nucleotides.--

<sup>3</sup>  
~~--70.~~ The method of claim <sup>1</sup>~~68~~, wherein said  
oligodeoxyribonucleotide comprises about 23 nucleotides.--

<sup>4</sup>  
~~--71.~~ The method of claim <sup>1</sup>~~68~~, wherein said targeted  
protein is follicle stimulating hormone, which has an alpha  
chain and a beta chain.--

*Sub. I'*  
*2*  
~~--72.~~ The method of claim <sup>1</sup>~~71~~, wherein the  
oligodeoxyribonucleotide comprises the nucleotide sequence <sup>from 3' to 5' of</sup>  
GTGTAGCAGTAR<sub>1</sub>CCR<sub>2</sub>GCGCACCA, and wherein R<sub>1</sub> is G or T and  
R<sub>2</sub> is G or T.--

<sup>16</sup>  
~~--73.~~ The method of claim <sup>1</sup>~~68~~, wherein said

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hybridization occurs at about 37°C.--

<sup>7</sup>  
--74. The method of claim <sup>1</sup>68, wherein said oligodeoxyribonucleotide is formed through diester bonding.--

--75. The method of ~~claim~~ 68, wherein said more stable form is a phosphotriester form.--

<sup>8</sup>  
--76. A method of controlling the infection of a host organism by a foreign organism through the selective inhibition of the synthesis of a protein vital to the foreign organism's viability, comprising the steps of:

5           determining the base sequence of the foreign organism's nucleic acid, said base sequence coding for at least a portion of said protein vital to the foreign organism's viability;

          synthesizing an oligodeoxyribonucleotide the order  
10 of nucleotides being substantially complementary to a portion of the foreign organism's messenger ribonucleic acid coding for said protein vital to said foreign organism's viability,

          at least a portion of said oligodeoxyribo-  
          <sup>in the form of a phosphotriester</sup>  
7 nucleotide being ~~a more stable form~~ to inhibit degradation in  
15 vivo;

          introducing said oligodeoxyribonucleotide into the cells of said host organism; and

          hybridizing said oligodeoxyribonucleotide with said portion of the foreign organism's messenger ribonucleic acid

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so as to substantially block translation of said foreign organism's messenger ribonucleic acid coding for said protein, thereby inhibiting synthesis of said protein vital to the viability of the foreign organism.--

--77. The method of ~~claim~~<sup>76</sup>, wherein said more stable form is a phosphotriester form.--

<sup>9</sup>  
--78. The method of claim ~~76~~<sup>8</sup> further comprising the step of:

determining the order of the base sequence of said organism's nucleic acid prior to synthesizing the oligodeoxyribonucleotide.--

<sup>10</sup>  
--79. The method of claim ~~76~~<sup>8</sup> further comprising the step of:

cross-hybridizing the oligodeoxyribonucleotide against messenger ribonucleic acid from at least one species different from said foreign organism and selecting that fraction of the oligodeoxyribonucleotide which does not so hybridize so as to increase the specificity of the selected oligodeoxyribonucleotide against said foreign organism.--

<sup>11</sup>  
--80. The method of claim ~~79~~<sup>10</sup>, wherein said cross-hybridization is performed against messenger ribonucleic acid from said host organism.--

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<sup>12</sup>  
--81. The method of claim ~~49~~<sup>10</sup>, wherein the selected oligodeoxyribonucleotide substantially hybridizes only with a messenger ribonucleic acid unique to said foreign organism.--

<sup>13</sup>  
--82. A genetically engineered therapeutic process which inhibits synthesis of one or more targeted proteins within the cells of an organism without substantially inhibiting synthesis of non-targeted proteins, comprising the steps of:

determining the base sequence of the messenger ribonucleic acid coding for the targeted protein;

synthesizing an oligodeoxyribonucleotide having a nucleotide sequence substantially complementary to the region of the messenger ribonucleic acid coding for said targeted protein,

at least a portion of said oligodeoxyribonucleotide being <sup>in the form of a phosphotriester</sup> ~~a more stable form~~ to inhibit degradation in vivo;

introducing said oligodeoxyribonucleotide into the cells of said organism; and

hybridizing said oligodeoxyribonucleotide with said base sequence of said messenger ribonucleic acid coding for said targeted protein, whereby translation of said base sequence is substantially blocked and synthesis of said targeted protein is inhibited.--

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REMARKS

This application has been carefully reviewed in light of the Office Action dated November 27, 1985. In that Office Action, the Examiner withdrew the finality of the Office Action mailed May 8, 1985, in light of newly cited art. The Specification was objected to and claims were rejected under 35 USC 112 and/or 35 USC 103 or 35 USC 102(e). In response, claims 1-39 have been cancelled and claims 54-82 substituted therefor. In this connection, revisions to the claims conform to those submitted to the Examiner in an interview on October 23, 1985, and reflect a number of helpful suggestions made by the Examiner at that time.

The Examiner's time and attention with respect to this application is greatly appreciated. Applicant wishes to particularly thank the Examiner for his courtesies during the recent interview, at which time various aspects of the previous Office Action were discussed. Those present at the interview of October 23, 1985, were Examiner Martinell, the applicant, Mr. Richard Tullis; his representatives, Mr. Gilbert Kovelman and Ms. Cathryn Campbell; and Mr. Vincent Frank, President of Molecular Biosystems, Inc., the assignee of the present application. At the interview, the invention was discussed in detail, and a proposed set of new claims, conforming to those hereinafter introduced by amendment, were provided to the Examiner.

As the new claims were intended to avoid points raised by the Examiner in the previous Office Action, they were discussed in some detail. In particular, the claims

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were limited to deoxyribonucleotides, which limitation the Examiner stated to have obviated previous rejections under 35 USC 112 which should be withdrawn. Moreover, the claims have been limited to oligodeoxyribonucleotides binding to mRNA sequences in the coding region so as to permit inhibition of synthesis of specific targeted proteins. The Examiner indicated that he was favorably impressed with this argument as distinguishing over the prior art. In light of the fact that the Examiner introduced new art, viz Miller et al. U. S. Patent No. 1,151,713, the finality of the previous rejection was withdrawn.

The latest Office Action again includes rejections duplicating those of the previous Office Action, which rejections were discussed at the Interview of October 23, 1985. In a recent telephone interview, the Examiner indicated to Ms. Campbell that the rejections were merely repeated for formal reasons.

In the latest Office Action, claims 1-4, 6-15, 18-25, 27-37, 39, and 53 were rejected, and the Specification objected to under 35 USC 112, first paragraph, as failing to provide an enabling disclosure. Specifically, it was asserted that the application does not provide evidence of the introduction of oligoribonucleotides into the cytoplasm of the cells with which it is contacted, as it must in order to form the RNA-RNA hybrids as allegedly specified and claimed.

As applicant has stated in previous papers, it is his belief that such uptake of oligoribonucleotides is clearly confirmed by various references, most notably Befort. In any event, however, the rejected claims have been

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cancelled and the new claims have been limited to oligodeoxyribonucleotides in accordance with the suggestion of the Examiner in the Office Action of May 8, 1985. This ground of rejection is thus believed to have been obviated. Moreover, the Examiner indicated at the Interview of October 23, 1985, that the newly cited Miller reference, U. S. Patent No. 1,151,713, corroborates uptake of oligonucleotides by cells.

Claims 1-4, 6-15, 18-25, 27-37, 39, and 53 were rejected under 35 USC 112 as the specification is asserted not to be enabling for RNA-RNA hybridization. As noted above, the rejected claims have been cancelled. The substituted claims are limited to deoxyribonucleotides and, thus, it is again believed that this ground of rejection is obviated.

Claims 14, 16, 17, and 27 were rejected under 35 USC 112, second paragraph, for lacking antecedent basis for certain claims. Again, in light of the amendment herein, these informalities have been overcome.

Claims 1 and 3-19 were rejected under 35 USC 103 as being unpatentable over Itakura et al. in view of either Paterson et al. or Hastie et al. All claims were also rejected under 35 USC 103 in view of the above references and also in view of any one of Pluskal et al, Pitha (CRC Press) , Befort et al., Arya et al. (Molec. Pharmacol. or BBRC), Summerton, Tennant et al., Miller et al. (Biochem. 16:1988), Stephenson et al., Zamecnik et al., or Stebbing et al. The Examiner has previously alleged that Itakura discloses the synthesis of oligonucleotides and the secondary and tertiary references disclose inhibition of protein synthesis and the

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introduction of oligonucleotides into cells. In his Amendment dated December 4, 1984, applicant has previously presented references and arguments indicating that the present invention is not rendered obvious by the cited references. Applicant will not repeat this material here in light of the present amendment which restricts all claims to oligodeoxyribonucleotides which are introduced into cells so as to inhibit protein synthesis. As has been previously stated, neither alone nor in combination do the cited references disclose or suggest the introduction of oligonucleotides complementary to specific portions of the coding region of an organism's mRNA so as to inhibit synthesis of the particular targeted proteins. Moreover, in the claims, as now amended, these restrictions are explicit, an argument with which the Examiner indicated his favorable impression at the Interview of October 23, 1985.

All claims have been rejected under either 35 USC 102 or 35 USC 103 on the basis of newly cited art, namely U. S. Patent No. 4,469,863 to Ts'o et al. (It is assumed that the Office Action's listing of claim 43 was a typographical error, and should have read claim 53). The Examiner alleges that Ts'o teaches specific inhibition of protein synthesis by using oligonucleotides complementary to mRNA. Applicant respectfully traverses this ground of rejection.

The Ts'o reference involves synthetic oligonucleotide aryl and alkyl phosphonates for use primarily in intracellular binding to transfer RNA anticodons or to transfer RNA amino acyl acceptor stems. Transfer RNA's so bound at the anticodon site are either unable to reach amino

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acids or are unable to recognize and bind to the complementary codon of the messenger RNA and thus protein synthesis is allegedly inhibited.

While the reference purports to teach that such tRNA binding can be used to accomplish specific inhibition of a selected nucleotide sequence so as, for example, to inhibit the growth of tumor cells of viruses, the examples specifically teach away from the use of these oligonucleotides to inhibit in vivo protein synthesis, as taught specifically in the present application.

Ts'o tested the effect of short homooligo-A deoxyadenosine (d-A<sub>2-4</sub>) methyl phosphonate in bacterial and mammalian cells and cell-free systems. In the cell free system, while some effect was noted on synthetic poly-A and poly-U messages, significantly no effect was observed on the translation of globin mRNA. The rabbit beta-globin polypeptide has some 3 phenylalanine residues coded for by the triplet U-U-U. In addition, there are seven other positions in beta-globin mRNA to which the tetrameric methyl phosphonate should bind. Therefore, were Ts'o's oligonucleotides truly capable of combining with the message so as to inhibit translation, an effect on globin synthesis would be observed. The fact that the inventors detected no such effect suggests that, in fact, these oligonucleotides are incapable of blocking translation of the message. Moreover, the fact that significant effects were observed on synthetic poly-A as well as poly-U mRNA suggests some sort of competitive interaction with tRNA rather than specific hybridization with the homopolymer template.

In testing their methyl phosphonate oligonucleotides

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on mammalian cells, Ts'o was able to establish uptake, as taught in the present application. Further, inhibitory effects on colony formation were observed in both bacterial and mammalian cells in culture. However, significantly, the inventors note at Column 26, lines 5-7 that "no inhibitory effects in cellular protein or DNA synthesis could be detected in the presence of [the stabilized oligonucleotides] by the present assay procedures."

Several explanations for the effect of the methyl phosphonate oligonucleotides are proposed. As Ts'o states at Column 27, lines 17-19, their observations "suggest that inhibition [of cell-free aminoacylation] occurs as a result of oligomer binding to the -- -U-U-U -- anticodon loop of the tRNA." It is noted that the results presented are kinetically consistent with interaction with the anticodon loop sequence. Moreover, because of evidence that the anticodon loop of the lysine tRNA in E. coli is related to the synthetic recognition site, the effect may derive from interference with the coupling of the amino acid to the corresponding transfer RNA.

Thus, it is apparent that in contrast to the Examiner's suggestion that methyl phosphonate oligonucleotides in Ts'o could be utilized to specifically control and inhibit protein synthesis in vivo, the experimental evidence presented in the Ts'o specification actually teaches away from such a use. While the data is consistent with methyl phosphonate oligonucleotides binding anticodons, nowhere do the results indicate that the oligonucleotides are selectively binding with the portion of the mRNA coding for specific proteins. In fact, the data in